REMARKS

Claims 42 to 66 and 83 to 89 are pending in this application. Claims 44, 47, 49 to 61, 65 and 66 have been withdrawn. In view of their withdrawal from consideration, claims 44, 47, 50 to 61, 65 and 66, directed to non-elected subject matter, have been canceled without prejudice. In addition, without conceding to the propriety of the rejections and in order to expedite prosecution of the application, claims 42, 43, 45, 47, 50-61 and 85 to 89 have been canceled without prejudice, claims 46, 48, 49, 62 to 64, 83 and 84 have been amended, and new claims 90 to 95 have been added. Applicant reserves the right to prosecute the subject matter of the canceled claims as well as the subject matter deleted from the amended claims in the present application and/or one or more related applications.

Claim 46 has been rewritten in independent form. Claims 48, 49, 62, 63, 83 and 84 have been amended to change the dependency of the claims. Claims 62 and 64 have also been amended to revise the language of the claims. New claims 90 to 92 are directed to similar subject matter as claims 62 to 64, but depend from claim 49 rather than claim 48. New claims 93 to 95 recite the location of the tRNA intron within the nucleic acid substrate. The claims are supported by the specification of the application as originally filed, *see, e.g.*, page 9, lines 20-21; page 10, lines 3-31; page 11, lines 7-32; and page 28, lines 23-27; and thus, does not constitute new matter.

Accordingly, after entry of the present Amendment, claims 46, 48, 49, 62 to 64, 83, 84 and 90 to 95 will be pending in the present application.

I. The Claim Objections Should Be Withdrawn

Claims 48 and 63 are objected to because they depend upon withdrawn claims. In view of the cancellation of previously withdrawn claims 47, 53 and 59, upon which claims 48 and 63 depend, Applicant has amended the dependency of claims 48 and 63. In particular, claim 48 has been amended to only depend from claim 46 and claim 63 has been amended to depend from claims 46 and 48. Accordingly, Applicant respectfully requests that this objection be withdrawn.

II. The Rejections under 35 U.S.C. § 103(a) Should Be Withdrawn

Claims 42, 43, 45, 46, 48 and 62-64 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Tocchini-Valentini et al., International Publication No. WO 01/92463 ("Tocchini-Valentini"), in view of Gontarek, International Publication No. WO 00/67580 ("Gontarek"). Claims 42, 43, 45, 46, 48, 62-64 and 87-89 are rejected under 35

U.S.C. 103(a) as allegedly being unpatentable over Tocchini-Valentini, Gontarek in view of Abelson et al., 1998, Journal of Biological Chemistry 273: 12685-12688 ("Abelson"). For the reasons of record and as well as those below, the rejections under 35 U.S.C. 103(a) should be withdrawn.

As acknowledged by the Examiner, Tocchini-Valentini does not teach or suggest a method that includes "assaying for a compound that can reduce (or inhibitor *[sic]*) RNA splicing as recited in claims 42, 43, 46 and 48", or "a fungal cell such as a yeast cell recited in claims 46, 63 and 64" (see November 12, 2009 Office Action at p. 6). Rather, as stated in Applicant's previous response of August 17, 2009, Tocchini-Valentini describes *utilizing the characteristics of the first step of the tRNA splicing reaction* (*i.e.*, cleavage of the pre-tRNA substrate), particularly the specificity of cleavage, to develop a *method of cleaving a target RNA molecule* that has a bulge-helix-bulge conformation by exposing said RNA to a eukaryotic or archael tRNA splicing endonuclease. Tocchini-Valentini describes the use of the RNA cleavage method for the removal of unwanted RNA molecules from cells (see p. 9, paragraph [0031]), the detection of the presence of particular types of RNAs (see p. 9, paragraph [0030]), the cleavage of a target RNA into defined ends with a 2',3' cyclic phosphate capable of being ligated (see p. 8, 9, paragraph [0029]), as well as the degradation of particular targeted RNAs (see p. 9, paragraph [0029]). Hence, Tocchini-Valentini, in its entirety, does not provide any hint or suggestion that eukaryotic tRNA splicing endonuclease can be established as a drug target used for screening for compounds that modulate the ability of fungal tRNA splicing endonuclease to produce mature tRNA.

Moreover, Tocchini-Valentini does *not* teach or suggest a nucleic acid substrate comprising the coding region of a reporter gene and a tRNA intron in a mature domain of a precursor tRNA, wherein the tRNA intron is contained within the nucleic acid substrate such that the mRNA transcribed from the coding region of the reporter gene is out of frame, much less the use of such a substrate in a method to screen for compounds that modulate the ability of fungal tRNA splicing endonuclease to produce mature tRNA. Instead, Tocchini-Valentini describes the use of a target molecule containing a bulge-helix-bulge conformation as a substrate for eukaryotic tRNA splicing endonuclease in methods for cleaving the target molecule. In fact, Tocchini-Valentini states that the cleavage of the bulge-helix-bulge conformation by the tRNA splicing endonuclease is "in the absence of the mature domain of the tRNA structure or sequence" (Tocchini-Valentini at page 7, paragraph [0024]). Nowhere does Tocchini-Valentini teach or suggest a method for screening for compounds that modulate the ability of any tRNA splicing endonuclease to produce mature tRNA using a

bulge-helix-bulge-containing substrate, much less a method for screening for compounds that modulate the ability of a fungal tRNA splicing endonuclease to produce mature tRNA using a nucleic acid substrate comprising the coding region of a reporter gene and a tRNA intron in a mature domain of a precursor tRNA as recited in the claimed methods. Accordingly, nowhere does Tocchini-Valentini teach or suggest the claimed methods.

Even assuming *arguendo* that one skilled in the art would have been motivated to use a nucleic acid substrate comprising the coding region of a reporter gene and a tRNA intron in a mature domain of a precursor tRNA, one skilled in the art would *not* have had a reasonable expectation of successfully cleaving such a substrate in an *in vivo* reporter gene assay to produce a full length protein in view of the results described in Tocchini-Valentini.

First, Tocchini-Valentini reports that in an *in vivo* reporter assay, a eukaryotic tRNA endonuclease does not recognize and cleave a reporter gene containing a bugle-helix-bulge structure. Tocchini-Valentini demonstrates that in an *in vivo* reporter gene assay that relies on an endogenous eukaryotic tRNA splicing endonuclease (in this case, mouse tRNA splicing endonuclease) for cleavage of a reporter gene containing a bulge-helix-bulge structure, a full length protein is not produced (see Example 3 of Tocchini-Valentini at pages 24 to 25, in particular paragraphs [0076] and [0077]). Tocchini-Valentini states that "Fig. 6 shows that if 3T3 cells are transiently cotransfected with a plasmid expressing GFP of [*i.e,* Green Fluorescent Protein reporter gene containing a canonical bulge-helix-bulge that renders the mRNA of the GFP out of frame] and a plasmid expressing the *M. jannaschii* endonuclease, GFP is produced. If a plasmid expressing an inactive endonuclease is utilized [*i.e.*, inactive *M. jannaschii* endonuclease], GFP is not produced." (see p. 25, paragraph [0077]). These results demonstrate that the endogenous eukaryotic tRNA splicing endonuclease does *not* cleave the reporter gene containing the bulge-helix-bulge structure and that cleavage of this reporter gene is dependent on an exogenously provided archael tRNA splicing endonuclease. According to Tocchini-Valentini, the position of the bulge-helix-bulge structure is irrelevant because the results from the *in vivo* reporter gene were similar whether the bulge-helix-bulge structure was inserted in the coding region of the GFP reporter gene or in the 3' untranslated region (see p. 26, paragraph [0079] and Figs. 9 and 10).

Second, Tocchini-Valentini reports that in an *in vitro* assay eukaryotic tRNA splicing endonuclease can recognize and cleave a bulge-helix-bulge structure (Tocchini-Valentini at, *e.g.*, page 23, paragraph [0070]). Tocchini-Valentini concludes that "eukaryal endonucleases retain the ability to operate in the mature-domain independent mode when their natural

substrates do not have a BHB" (Tocchinin-Valentini at page 24, paragraph [0071]; emphasis added). Thus, in view of the *in vitro* results reported in Tocchini-Valentini, one skilled in the art would have expected that similar results would be obtained in the *in vivo* reporter gene assay described in Tocchini-Valentini whether a nucleic acid substrate containing either a bulge-helix-bulge conformation or a tRNA intron in a mature domain of a precursor tRNA was used as the substrate.

Accordingly, in view of the *in vitro* and *in vivo* results reported in Tocchini-Valentini, one skilled in the art would not have expected that a eukaryotic tRNA splicing endonuclease would cleave a nucleic acid substrate comprising the coding region of a reporter gene and a tRNA intron in a mature domain of a precursor tRNA as recited in the claimed methods to produce a full length protein.

Since the reporter gene assay of the claimed methods is based, at least in part, upon the ability of the fungal tRNA splicing endonuclease (a eukaryotic tRNA splicing endonuclease) to cleave a tRNA intron in a mature domain of a precursor tRNA, one of ordinary skill in the art would *not* have had a reasonable expectation of identifying a compound that modulates the ability of fungal tRNA splicing endonuclease to produce mature tRNA in view of the results in Tocchini-Valentini. Instead, in view of the results described in Tocchini-Valentini, one of ordinary skill in the art would have expected that the fungal tRNA splicing endonuclease would *not* cleave a tRNA intron in a mature domain of a precursor tRNA found in a nucleic acid substrate containing the coding region of a reporter gene, and thus, the protein translated from the mRNA of the coding region of the reporter gene would not be full length. Therefore, one of ordinary skill in the art would *not* have had a reasonable expectation of identifying a compound that modulates the ability of a fungal tRNA splicing endonuclease to produce mature tRNA according to the claimed methods which rely on the ability of the fungal tRNA splicing endonuclease to cleave an intron in a mature domain of a precursor tRNA.

Nothing in Gontarek or Abelson, alone or in combination, teaches or suggests the claimed methods, much less provides one of ordinary skill in the art with a reasonable expectation of success. Gontarek relates to methods of screening for compounds that modulate fungal *pre-mRNA* splicing, not methods for identifying a compound that modulates the ability of fungal *tRNA* splicing endonuclease to produce mature tRNA, which is the subject matter of the instant claims. As Applicant has previously argued, there are fundamental differences between mRNA splicing and tRNA splicing that would *not* suggest

to one of ordinary skill in the art to substitute one splicing pathway for another.

Although both mRNA splicing and tRNA splicing involve the removal of introns, the mechanism of action for the removal of introns from pre-mRNA is very different from the removal of introns from pre-tRNA (Abelson *et al.*, 1998, J Biol Chem., 273:12685-688 ("Abelson"); see p. 12685, col. 1, first paragraph). It is well known in the art that the substrates, mechanism of action and factors involved in the mRNA and tRNA splicing pathways are completely different. For example, mRNA splicing occurs in the nucleus via the spliceosome, a large macromolecular complex of five small nuclear RNA proteins and protein factors that catalyze the efficient and accurate removal of introns from mRNA (Gontarek at page 1, lines 7-8), whereas tRNA splicing occurs in the cytoplasm and requires three different enzymes, ATP hydrolysis and has different substrates than mRNA splicing. Thus, Gontarek does not teach or suggest the claimed methods.

Moreover, given the results reported in Tocchini-Valentini using a eukaryotic tRNA splicing endonuclease, one of ordinary skill would *not* have had a reasonable expectation of identifying a compound that modulates the ability of a fungal tRNA splicing endonuclease to produce mature tRNA by combining the disclosure in Gontarek regarding pre-mRNA splicing assays with the disclosure in Tocchini-Valentini regarding tRNA splicing endonuclease and BHB-containing substrates.

Abelson is a review article regarding tRNA splicing, the focus of which is archael tRNA splicing endonuclease of *M. jannaschii* and a comparison with eukaryal tRNA splicing endonuclease. Although Abelson mentions that the mature domain of precursor tRNA is involved in eukaryotic tRNA splicing endonuclease, there is no teaching or suggestion in Abelson to develop a reporter gene assay such as described in the claimed methods. Furthermore, as discussed above, given the results described in Tocchini-Valentini, one of ordinary skill in the art would *not* have had a reasonable expectation of identifying a compound that modulates the ability of a fungal tRNA splicing endonuclease to produce a mature tRNA utilizing a nucleic acid substrate comprising the coding region of a reporter gene and a tRNA intron in a mature domain of a precursor tRNA as recited in the claimed methods.

The disclosure in International Publication WO 01/25486 by Rana ("Rana") does not cure the deficiencies of Tocchini-Valentini, Gontarek or Abelson. Rana does not teach or suggest the claimed methods, much less provide a reasonable expectation of success.

Rana does not teach or suggest screening for compounds that *modulate the activity of*

an enzyme, much less modulation of the ability of a fungal tRNA splicing endonuclease to produce mature tRNA. Rana only discusses *compounds that bind to RNA*. Thus, contrary to the Examiner's allegation, one of ordinary skill in the art would *not* have been motivated to modify the methods of Rana for identifying compounds that bind to a target RNA molecule in order to develop a method for identifying compounds that modulate the ability of a fungal tRNA splicing endonuclease to produce mature tRNA. Moreover, given the results for the reporter gene assay described in Tocchini-Valentini, alone or in combination with Abelson, Gontarek and Rana, one of ordinary skill in the art would *not* have had a reasonable expectation of identifying a compound that modulates the ability of fungal tRNA splicing endonuclease to produce mature tRNA as claimed.

In view of the foregoing, the rejections under 35 U.S.C. § 103(a) should be withdrawn.

III. The Rejection Under 35 U.S.C. § 112 Should Be Withdrawn

Claims 83-86 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. Claims 85 and 86 have been canceled. Therefore, the rejection of claims 85 and 86 is rendered moot. For the reasons provided below, the rejection of claims 83 and 84 should be withdrawn.

The substrate recited in claims 83 and 84 is considered to be the same substrate for both the animalia and the fungal tRNA endonuclease. The specification states that "'a substrate for a tRNA splicing endonuclease' refers to any nucleotide sequence recognized and excised by a *eukaryotic* tRNA splicing endonuclease. For example, a nucleotide sequence comprising a bulge-helix-bulge structure or a mature domain of a precursor tRNA may be utilized as a substrate for a fungal tRNA splicing endonuclease..." (*see* specification, p. 28, lines 22-26; emphasis added). It is well know to a person skilled in the art that at the time of filing, both animalia and fungal tRNA endonucleases have the same substrate. In Trotta and Abelson, 1999, Cold Spring Harbor Press, p. 561-584 ("Trotta and Abelson", cited as Reference C29 in the Information Disclosure Statement filed on July 20, 2007), the authors teach that "[l]ike the yeast tRNA introns, the position of the introns in higher eukaryotes is conserved and the cleavage reaction operates in a manner identical to the yeast tRNA splicing endonuclease as exemplified by *Xenopus* endonuclease." (see Trotta and Abelson, p. 569, third paragraph). Accordingly, the substrate recited in claims 83 and 84 is considered to be the same substrate for both the animalia and the fungal tRNA endonuclease. Therefore, the specification *does* provide support for using the same substrate for both the animalia and the

fungal tRNA splicing endonucleases.

In view of the foregoing, the rejection of claims 83 and 84 under 35 U.S.C. § 112, first paragraph should be withdrawn.

IV. The Double Patenting Rejection Should Be Held In Abeyance

Claims 42, 43, 45, 46, 48, 62-64 and 83-89 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 55-57, 63-74, 76-82 and 91-95 of co-pending Application No. 10/551,301, in view of Tocchini-Valentini et al., International Publication No. WO 01/92463.

This is a *provisional* obviousness-type double patenting rejection because the conflicting claims have not in fact been patented. Accordingly, Applicant respectfully requests that this rejection be held in abeyance until such time as there is allowable subject matter.

CONCLUSION

Applicant believes that the present claims meet all of the requirements for patentability. Consideration and entry of the amendments and remarks made herein into the file history of the present application are respectfully requested. The Examiner is invited to contact the undersigned if any issues remain.

Respectfully submitted,

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